VOLUME - 10, ISSUE - 10, OCTOBER - 2021 • PRINT ISSN No. 2277 - 8160 • DOI : 10.36106/gjra

Original Research Paper

Microbiology

RAPID TEST FOR DETECTION OF CARBAPENAMASES IN BURN PATIENTS DURING COVID 19 PANDEMIC

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ABSTRACT

BACKGROUND: Infection is the leading cause of death after extensive burn injuries even with advancements in burn care over the last 50 years. About 42%–65% of deaths in burn victims are attributable to infection caused by multidrug resistance amongst Gram-negative bacteria (GNB). The prevalence of multidrug-resistant (MDR) bacteria in burn centers may result in the empiric selection of antibiotics that target MDR bacteria, thus propagating a vicious cycle of increased antimicrobial resistance during COVID-19 times.

OBJECTIVE: To assess the utility of Rapidec Carba NP test as screening test to detect Carbapenemase production in Gram Negative bacilli in burn patients.

METHODS AND MATERIAL: A total of 150 pus/wound samples were processed from November 2019 to December 2020 of burn patients and were screened by Rapidec Carba NP test to detect carbapenem reistance.

RESULTS: A total of 89 *Enterobacterales* isolates were tested for carbapenemase production out of which 78 were carbapenamase resistant. 33 were MBL positive and then these isolates were tested by Rapidec Carba NP Test. Four MBL isolates were not detected by Rapidec Carba NP test giving sensitivity of 88%.

CONCLUSIONS: Rapidec Carba NP test has turnaround time of 30 minutes to 2 hours which proves as effective screening test for carbapenem reistance in burn patients.

KEYWORDS :

INTRODUCTION

Infection is now the leading cause of death after extensive burn injuries even with advancements in burn care over the last 50 years. As compared to uninfected patients, burn patients with infections have more than twice the mortality rate.[1] Over the last decade about 42%–65% of deaths in burn victims are attributable to infection caused by multidrug-resistant (MDR) bacteria which is spreading worldwide at an alarming rate amongst Gram-negative bacteria (GNB). Therefore the prevalence of MDR bacteria in burn centers results in the empiric selection of antibiotics to combat increased antimicrobial resistance.[2]

While GNB have developed several mechanisms to avert the bactericidal effects of commonly prescribed antibiotics but, it has been noticed that there is increase in prevalence of carbapenemase-producing organisms (CPO). This is of worrisome because of the resistance mechanisms as well as these are the last resort of treatment left in hand. The resistance mechanism includes the the rapid spread of mobile genetic elements carrying carbapenemase genes, a decrease in bacterial outer membrane permeability with overexpression of AmpC/ESBL, or overexpression of efflux pump. As it is known that carbapenemases are specific beta lactamase present in GNBs, with ability to hydrolyse carbapenem, the widespread use of carbapenemases in clinical practice has led to the development of resistance to these antibiotics and therefore carry high mortality amongst the burn patients.[3] Recently decreased susceptibility to carbapenems has been increasingly reported worldwide in *Enterobacteriaceae, Pseudomonas spp., and Acinetobacter spp.* [4]

Carbapenemase-producing *Enterobacterales* (CPE) are a rising threat to global health aswell. Infections with CPE are not only associated with increased mortality but also with nosocomial transmission in hospitals.[5]

Metallo-β- lactamase (MBL) are zinc dependant β- lactamases which hydrolyse all β lactamase except aztreonam.[6] In 2008 NDM1(New Delhi Metallo β lactamase) was discovered from India. Even NDM1 positive isolates carries additional resistance mechanism esp to aminoglycosides, fluoroquinolones group thereby further transferring into another species resulting in wider spread of antibiotic resistance and narrowing the treatment options in burn patients. [7]

The number of tests available for detection of carbapenemase are classified as biochemical, phenotypic and molecular methods. The non-molecular methods are immuno chromatographic assays, colorimetric tests, the carbapenem inactivation method and modifications thereof, and matrix-assisted laser desorption ionization-time of flight MS-based tests. The tests vary widely in accuracy and turnaround time.[8]

Phenotypic Screening of MBL is very useful to limit the spread of MBL infections in burn wards, in case of early detection, infection control measures like barrier precautions can be initiated in burn units without delay and also helps in selecting the apt antibiotic regimen in these patients to further decrease morbidity and mortality. So, early detection of CPE results in the containment of the spread of resistance and can be life-saving in patients with burns so as to prevent invasive infections.[9]

Based on the principle of acidimetry and developed by Nordmann et al, a novel carbapenemase detection test i.e Rapidec Carba NP (CNP) test hydrolysis the beta-lactam ring which results in a reduction in pH, causing a color change of indicator phenol red from red to yellow.[10]

The Clinical and Laboratory Standards Institute (CLSI) with a few modifications recommended the CNP test as a confirmatory test for carbapenemase production. The present study aimed to detect carbapenemases production in Enterobacterales by using a single protocol the Rapidec Carba NP test providing rapid results with good reliability in burn patients. After preparation of sample preparation, it showed a sensitivity and specificity of 96% in less than 2 hours. This ready-to-use test is well adapted to the daily need for

detection of carbapenemase to avoid invasive infection.[11]

Material and methods: This study was carried out in the Department of Microbiology, Government Medical College Hospital, Chandigarh, between September 2019 and December 2020. A total of 150 samples were recieved from burn patients during this period. Of 150 samples, the 161 bacterial isolates were identified to species level according to standard microbiological procedures. The antibiotic susceptibility testing for the same was done by Kirby-Bauer disk diffusion test following CLSI guidelines. The following drugs (Hi-media, Mumbai) were tested for *Enterobacterales;* ceftriaxone (30μg), cefotaxime (30μg), cefepime(30μg), amikacin (10μg), ciprofloxacin (10μg). The second line antibiotic susceptibility was done for piperacillin-tazobactam (100/10μg), imipenem (10μg) and meropenem (10μg). Those strains which showed reduced susceptibility (≤19 mm) based on disc diffusion test to meropenem/imipenem were confirmed for carbapenem resistance by microbroth dilution as per CLSI (MIC≥4μg/ml).

All these isolates were confirmed for MBL production as follows.

MBLs detection by disc diffusion test- Combined Disk test is done using Imipenem (10μg) & Imipenem+ ethylene diamine tetra acetic acid disc (Imipenem10μg+EDTA750μg HIGH-MEDIA). When the Zone difference of combined disk i.e. Imipenem+EDTA & Imipenem alone was >7mm, it was considered as production of MBL.

These isolates were then tested by Rapidec Carba NP test kit (BioMerieux SA, France) as follows;

Requirement- Test strains,10–100 µl pipette and Sterile tips Test procedure as suggested by manufacturers on the testing kit was

Step 1: Add 100 µl suspension given with kit in well "a," "b," "c" for rehydration of well for 5–10 min with lid covered at room temperature.

Step 2: Mix the content of well "b" with stirrer

Step 3: Add bacterial colonies into well "c" with same turbidity of well "b" and then cover with lid for 30 min at room temperature

Step 4: Transfer 25 µl from well "c" to both well "d" and well "e"(well "d" is control well and well "e" is test well)

Step 5: Transfer 25 µl from well "a" to both well "d" and well "e"

Step 6: Cover the lid and incubate it for 30 min at 37°C

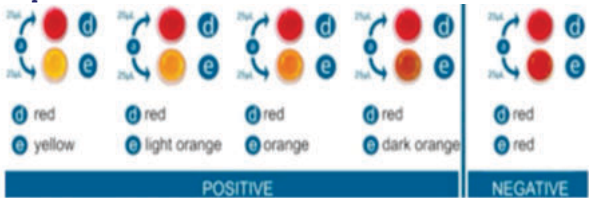

Figure 1 Shows the interpretation as shown by manufacturers on the testing kit.

Test interpretation- Reading should be taken after 30 min. A test is positive when a significant variation in color is observed between the two wells.If positive for carbapenemase production well "d" changes to red color and well "e" yellow to orange color as shown in Figure 1. For negative result second reading taken after 30 min.

RESULTS

Of the 161 bacterial isolates were identified out of which out of which *Pseudomona aeruginosa* (n=44), was the commonest followed by *Acinetobacter spp* (n=*15), Staphylococcus aureus* (n=*9) and* (n=*4) were CoNS*. Eighty nine isolates of *Enterobacterales* were selected for detection of Carbapenemase detection, of which (n=42) were *Klebsiella spp*, (n=35) isolates were *E. coli,* (n=3) were *Proteus spp and Citrobacter spp (n=9).* Out of total seventy eight carbapenemase positive isolates, seventy were detected by Rapidec CarbaNp test, giving sensitivity of (90%) for carbapenemase detection. Total thirty three (42.3%) were MBL positive. Out of thirty three phenotypically characterised MBL positive isolates, twenty nine isolates were detected by Rapidec CarbaNP test, with sensitivity of 88 % for MBL Class of carbapenemase, while four MBL positive isolates were not detected by Rapidec CarbaNp Test which was *Klebsiella pneumoniae* (n=3), followed by one *Proteus mirabilis* (n=1) isolate.

Among eleven carbapenemase sensitive strains, ten were detected as negative by Rapidec CarbaNP test whereas only one isolate was positive by the Rapidec CarbaNP test which was phenotypically negative, thus giving specificity of 100%.

DISCUSSION

This study was conducted on patients who suffered burns keeping in view their critical condition. Wounds are good media for bacterial growth in such patients. Soon after burn injury, colonization of bacteria happens which will continue to grow more and more. The more virulent strains have the potential to go deeper into the tissue and hence produce abscesses underneath. As there is no host defense, the bacteria will lodge into the adjacent soft tissues and then invade lymphatics and blood vessels, especially the venous vessels.[11] As multiorgan failure is also common with major burn injuries the reason being the generation of hypermetabolic/catabolic state which leads to immune system dysfunction leading to perpetuating systemic damage and worsening outcome even more in cases with CPE.[12]

Over time starting from penicillins the β-lactamase enzyme extended its activity which is followed by cephalosporinases, then producing ESBLs and recently even to the MBLs and other carbapenemases. The MBLs have hugely impacted the last resort of drugs esp, carbapenems for the management in burn patients.[13]

Although the leading infective bacterium in burn wounds is Staphylococcus aureus in the present study by Klebsiella spp was the most common among Perween et al showed the most frequently isolated Gram-negative bacteria from patients with burn wounds is Klebsiella spp which is comparable with the present study[14]

producers from strains that are carbapenem-resistant due to non–carbapenemase-mediated mechanisms, such as combined resistance mechanisms, outer-membrane permeability defect further associated with overproduction of cephalosporins and/or extended-spectrumβ-lactamases or from strains that are carbapenem susceptible but express an extended-spectrum β-lactamase, plasmid, and chromosome-encoded cephalosporinases. This test has multiple benefits from not very expensive, to rapid, more reproducible along with high sensitivity and specificity. It eliminates the need for using other techniques to identify carbapenemase producers that are time-consuming and less sensitive or specific. Using this accurate test would improve the detection of patients infected or colonized with carbapenemase producers. In addition, use of this test has greatly decreased the laboratory technicians' workload and simplified the clinical management of potential carbapenemase producers.[15]

One isolate of GNB which was phenotypically negative for MBL was also detected by Rapidec Carba Np test the most likely reason being incorporation of Zinc in reaction wells and this also aids in rapid detection of MBL positive isolates, in 5 to

10 minutes as also stated by Dortet et al.[16]

Also with 90% sensitivity & turnaround time of 30 minutes to 2 hours, Rapidec Carba NP test proves an effective screening test in burn patients. Interpretable positive results were obtained in <2 hours, making it possible to implement rapid containment measures to limit the spread of carbapenemase-producing organism in burn unit to decrease mortality [17]

Major or minor burn injuries during the COVID-19 pandemic can complicate the clinical presentation with a prolonged hospital stay in burn patients leading to financial crises. Developments in critical care and surgical approaches to treat burn wounds together with antimicrobial treatments have reduced the morbidity and mortality rates associated with this injury. With rising resistance and mere antimicrobials left in hand, it is needed for the hour to detect carbapenemases among Gram-negative bacteria (GNB) in burn patients for both the clinician as well as for infection control practices in the hospital. [18]

CONCLUSION

In summary, the spread of CPE remains a significant clinical and public health concern. Reliable detection of carbapenemase production is an essential to combat the problem globally. Therefore rapid screening and confirmatory test is essential for detection of cabapapenem resistance to improve overall outcome in burn patients which helps in reducing the burden of wrong consumption of antibiotics which can lead to disastrous effects on AMR management and antibiotic stewardship programmes.

LIMITATIONS

The limitatiom of the present study is that it is conducted on small patient group.

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